P-phenylenediamine

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CHAPTER 3

Real-time detection of *p*-phenylenediamine penetration in the human skin by *in vivo* Raman Spectroscopy

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Submitted

Abstract

Background Penetration, auto-oxidation and *N*-acetylation of *p*-phenylenediamine (PPD) has been studied *in vitro* and *ex vivo*. However, a clear understanding of *in vivo* PPD penetration and formation of PPD derivatives is lacking.

Objectives To get insight into *in vivo* penetration, clearance and formation of PPD (derivatives) in the human skin.

Methods Patch test chambers containing 1% PPD-petrolatum were applied to the forearms of 2 human volunteers, using increasing application times and several follow-up measurements. Non-invasive Raman Microspectroscopy was used for detection of PPD (derivatives).

Results Application a 1% PPD-petrolatum patch for 30 minutes resulted in substantial amounts of PPD in the stratum corneum of 90 mg PPD/g keratin. PPD contents were highest after repeated application of 3 times 1 h (350 mg PPD/g keratin), followed by single applications for 2 h 40 min, 2 h, and 23 h. The PPD half-time was 3 h. No spectral contributions of BB, MAPPD and DAPPD were detected.

Conclusions We have gained insight into the *in vivo* penetration of PPD in human skin by non-invasive Raman spectroscopy. Penetration into the skin was fast and detected PPD concentrations in the stratum corneum were high. PPD was detected in the stratum corneum as well as in the viable epidermis. Oxidized or acetylated PPD derivatives could not be detected.

Introduction

P-phenylenediamine (PPD) is intrinsically unstable and under air exposure or in solution it is susceptible to auto-oxidation and conjugation reactions. The highly reactive primary oxidation product of PPD, *p*-benzoquinonediimine (PBQD), is proposed to undergo oxido-conjugation reactions, to form dimers, the trimer Bandrowski's base (BB) or even pentamers.[1] In addition, several studies demonstrated that PPD is metabolized within the skin into monoacetyl-PPD (MAPPD) and diacetyl-PPD (DAPPD) by the enzyme *N*-acetyltransferase 1 (NAT1).[2] In the local lymph node assay (LLNA), these acetylation products have been shown to be non sensitizing [3] and therefore are considered detoxified products of PPD, while BB is classified as extreme sensitizer.[2,4] Nevertheless, an *in vivo* human elicitation study [4] suggests that BB may not play an important role in human sensitization to hair dyes. Furthermore, probably not PPD itself, but its reactive primary oxidation product PBQD, able to covalently bind to proteins and thereby to serve as hapten-protein complex, might be the key player.[5-7]

Despite all the experimental knowledge on PPD and on formation of its oxidation and acetylation products, a clear *in vivo* picture on how fast and to what concentrations PPD penetrates into the skin and on where and when these products are formed is lacking. Although *ex vivo* penetration experiments quantitatively studied the recovery of topically applied [^{14}C]PPD in a hair dye formulation in the different skin layers, no distinction was made between PPD, MAPPD, DAPPD or BB or other oxidation products.[8-11]

To study the real-time penetration of a topically applied chemical *in vivo*, non-invasive Confocal Raman Microspectroscopy (CRM) is the method of choice.[12] Briefly, CRM is an optical method in which a monochromatic laser light is focused onto the sample of interest, thereby exciting molecular vibrations and causing inelastic scattering of the light, called Raman-scattering. This scattered light has frequencies different from the incident light. The frequency shift is dependent on atomic mass, chemical bonds and molecular structure and therefore, the Raman spectrum is highly molecule specific. Consequently, a Raman spectrum represents a 'fingerprint' or signature by which a molecule can be identified, as well as quantified. The Raman spectrum of skin represents the sum of all Raman signals from the molecules present in the skin and thus provides a detailed picture of its molecular composition.[12] Once the Raman signature of a particular chemical has been obtained, and a sufficient amount penetrates the skin after topical application, the chemical can be identified and traced *in vivo* within the skin. This method has been used to study epidermal penetration of topical drugs (Metronidazole, *Trans*-Retinol) as well various other chemicals, including 2-butoxyethanol and toluene.[13-15]

This study was designed to answer the following questions: (i) 'What are the penetration characteristics of topically applied PPD as a function of depth in the skin and as a function of time?' and (ii) 'Can oxidation (BB) or acetylation products (MAPPD or DAPPD) be detected within skin after topical application of PPD?

Materials and methods

Sample preparation

P-phenylenediamine (PPD; CAS 160-50-3), *N*-Acetyl-*p*-phenylenediamine (MAPPD; CAS 122-80-5) and *N*-*N*'-diacetyl-*p*-phenylenediamine (DAPPD; CAS 140-50-1) were purchased from Sigma-Aldrich Chemie (Zwijndrecht, the Netherlands). Bandrowski's base (BB; CAS 20048-27-5) was purchased from Apollo Scientific Ltd (Stockport, United Kingdom). DMSO was ordered from Carl Roth (Karlsruhe, Germany). For *in vitro* determination of their reference spectra in solution PPD (1%) and MAPPD (1%) were dissolved in water and DAPPD (2%) and BB (1%) were dissolved in dimethylsulfoxide (DMSO). For spectral analysis, the spectra of the solvents were subtracted. For penetration studies 1% PPD-petrolatum and blank petrolatum were used (Trolab-Almirall Hermal, Reinbeck, Germany). For a quantitative calibration of the PPD concentration, solutions were prepared of 0.5-2.0% w/w PPD and of 0.5-5% w/w bovine serum albumin (BSA) in demineralized water. Bovine serum albumin was purchased from Sigma-Aldrich Chemie (Zwijndrecht, the Netherlands).

Volunteers

In vivo Raman spectra were obtained from 2 volunteers (1 female 30 years, 1 male 43 years, without history of PPD sensitization). The study was approved by the local ethics committee of the University Medical Center Groningen (UMCG), protocol number NL36149.042.11. The volunteers gave informed consent prior to participation. Experiments were conducted at the Department of Dermatology of the Erasmus MC, Rotterdam, the Netherlands.

Raman experiments

Experiments were performed using the Model 3510 Skin Composition Analyzer (RiverD International B.V., Rotterdam, the Netherlands) with 785 nm laser excitation, 25 mW laser power on the skin and a spatial depth resolution of 5 µm. For reference spectra, approximately 20 µl of the sample solution was pipetted into the cavity of a socket head cap screw and placed on the window. Petrolatum was directly applied onto the silica window. Signal collection times (up to 300 s) were chosen based on obtaining optimal signals.[16] For *in vivo* experiments approximately 20 mg of the 1% PPD-petrolatum formulation (Trolab®) was spread evenly on a van der Bend square patch test chamber covering a surface area of 0.64 cm^2 (Van der Bend, Brielle, the Netherlands) and applied to the volar forearm of the subject. A patch containing approximately 20 mg petrolatum served as a control. After removal of the patch, the skin was wiped once with a tissue and the imprint of the patch test square was marked at the borders. The marked location was placed on the silica window of the Raman device. By automatically moving the objective in the vertical position, the depth of the laser focus in the skin (Z) could be varied accurately. Raman profiles were recorded from Z = 0 µm (skin surface) to a maximum

of Z = 36 µm (viable epidermis), with increments of 2-4 µm and a signal collection time of 5 s per single spectrum.

Experiment 1: Penetration of PPD as a function of application time. A 1% PPD-petrolatum patch was applied on the forearm with different application times ranging from 30 min to 23 h. Additionally a 1% PPD-petrolatum patch was applied on the forearm for 3 h application time, during which the patch was replaced by a fresh patch every hour (indicated as "3 x 1 h"). Directly after removal of the patch, the skin was wiped clean and the distribution of PPD across the stratum corneum was measured by Raman spectroscopy. For all other application times a single patch was applied (Table 1).

Experiment 2: Clearance of PPD as a function of time. Clearance was assessed by repeated Raman measurements at different time intervals (2 h and 3 x 1 h) after removal of the 1% PPD-petrolatum patch. Raman measurements were performed 0-7 h after removal of the patch and once more after 27 h (Table 1). For each time interval all concentration profiles of PPD content versus depth were averaged and the area under the curve (AUC) from 0-20 µm below the skin surface was calculated with a standard trapezoid integration. The PPD clearance time (half-time) was determined as the time point at which the AUC had decreased by 50%.

Experiment 3: Detection of oxidation and/or acetylation products of PPD. In a separate experiment Raman spectra were recorded for a prolonged time (up to 60 min) at a fixed depth of 30 µm below the skin surface to maximize the total signal collection time per spectrum at given depth. This depth is in the viable epidermis, which is the expected location of the enzymatic acetylation of PPD.[16] In addition to these fixed depth measurements, Raman spectra obtained in experiment 2 were examined for the presence of BB, MAPPD or DAPPD and petrolatum.

Figure	**3**	**4**	**5**
Patch application time	0.5 h 2 h 2 h 40 min 3x1 h 23h	2 h	3x1 h
Number of volunteers	1-2	1-2	1-2
Start of measurements per experiment at t (h) after removal of the patch	Immediately (< 5 min)	0-0.5 h 0.5-1 h 1-2 h 2-3 h 4-5 h 6-7 h	0-0.5 h 0.5-1 h 3-3.5 h 6-6.5 h 27 h
Depth (µm)	0-32	0-24	0-36

Table 1 Overview of Raman measurements performed as displayed in figures 3-5.

Data analysis

The Raman instrument was calibrated according to the User Manual of the model 3510 SCA (RiverD International). Briefly, the spectrum of a neon-argon lamp in combination with an internal Raman standard were used to calibrate the spectrometer wavenumber axis and the NIST SRM 2241 Reference Material was used for the relative intensity correction of the spectrometer. Instrument background signals were subtracted from each measured spectrum. All spectra collected were analyzed using SkinTools 2.0 analysis software (RiverD International BV). The analysis consisted of fitting each spectrum collected from the skin with a fitting model based on the endogenous components of the skin.[12] Briefly, this model uses a classical least squares fit to determine the contribution of each of the reference skin components to the *in vivo* spectrum collected from the skin. *In vitro* reference spectra of PPD, blank petrolatum, BB, MAPPD and DAPPD were added to this fit model. This allows the generation of depth profiles of each added component and the reference skin component to be obtained. In CRM, the collected signal decreases when probing deeper into the tissue due to the loss of light by scattering. To compensate for this effect all fit coefficients were normalized on the keratin signal as an intrinsic standard, since keratin is the dominant protein and represents the majority of the dry weight of the stratum corneum. The intensity of the PPD Raman signal relative to the intensity of the keratin Raman signal was calibrated in order to determine the PPD content in the skin in mg/g keratin. For this calibration Raman spectra of aqueous solutions of BSA (0.5– 5% w/w) and of PPD (0.5–2%) were measured and normalized on the water signal. BSA was used because it is highly water-soluble and because the Raman spectrum of BSA and the intensity of the Raman signal are largely similar to those of keratin.[12] The normalized Raman spectra from the PPD and BSA solutions were then used to calculate the scale factors of the fit model spectra of PPD and of keratin, such that the ratio between the fit coefficients of PPD and keratin represents the mass ratio of PPD to keratin. In this way the PPD content, as calculated by the classical least squares fit, was expressed in mg PPD/g keratin. The limit of detection for PPD was defined as the calculated PPD content after application of a petrolatum patch without PPD. This residual value represents the error of the classical least squares fit model.

Results

The reference spectra of PPD, MAPPD, DAPPD and BB after subtraction of the Raman spectrum of the respective solvent are depicted in Figure 1. Spectra for petrolatum and untreated stratum corneum are also shown. PPD shows 4 characteristic Raman bands that clearly separate PPD from the endogenous skin constituents (Figure 1, characters a-d).

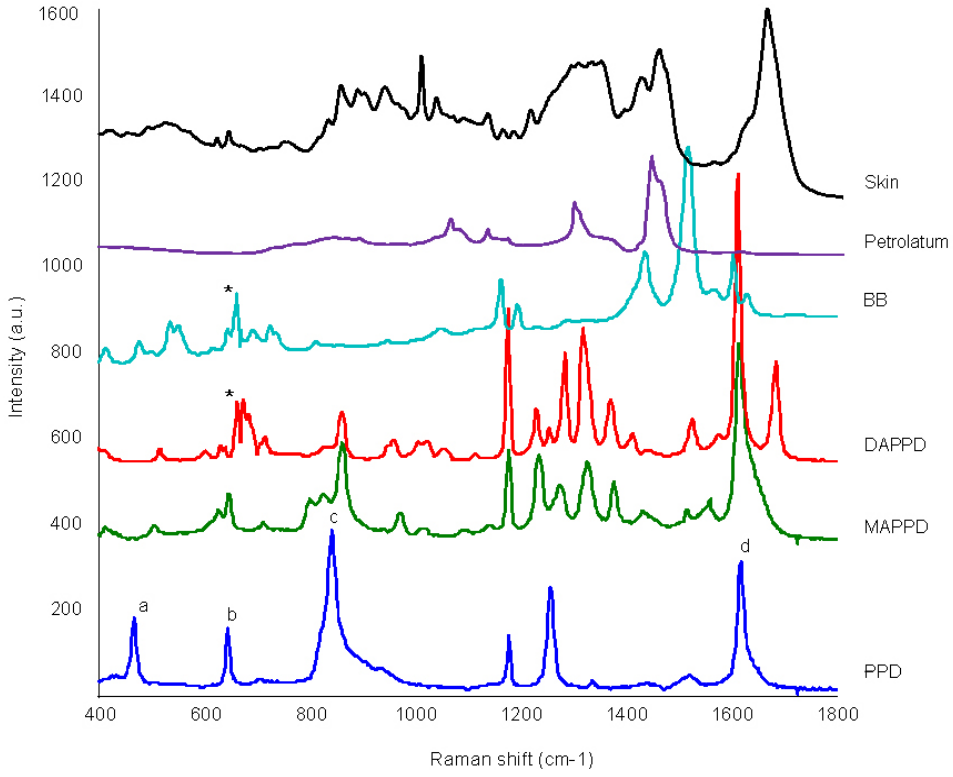

Figure 1 Raman reference spectra of *p*-phenylenediamine (PPD), monoacetyl-PPD (MAPPD), diacetyl-PPD (DAPPD), Bandrowski's base (BB), petrolatum and the skin. The peaks characteristic to PPD which are distinguishable from skin signals, can be found at (a) 470 cm^{-1}, (b) 648 cm^{-1}, (c) 844 cm^{-1} and (d) 1622 cm^{-1}. The asterix around 650 cm^{-1} in the spectra of BB and DAPPD indicate an artifact due to the subtraction of the solvent spectrum (DMSO).

Detection of PPD in the stratum corneum

A 1% PPD-petrolatum patch was applied to the skin of the volar forearm for 2 h 40 min. The untreated adjacent skin served as control. The PPD signal can be clearly distinguished in the *in vivo* Raman spectrum of skin (Figure 2). Shown are a spectrum of untreated control skin and a spectrum of skin after application of a 1% PPD-petrolatum patch. The 4 characteristic Raman bands of PPD were clearly discernible, indicating the presence of PPD within the stratum corneum. No signals for the auto-oxidation product BB or other PPD derivatives were detected within stratum corneum.

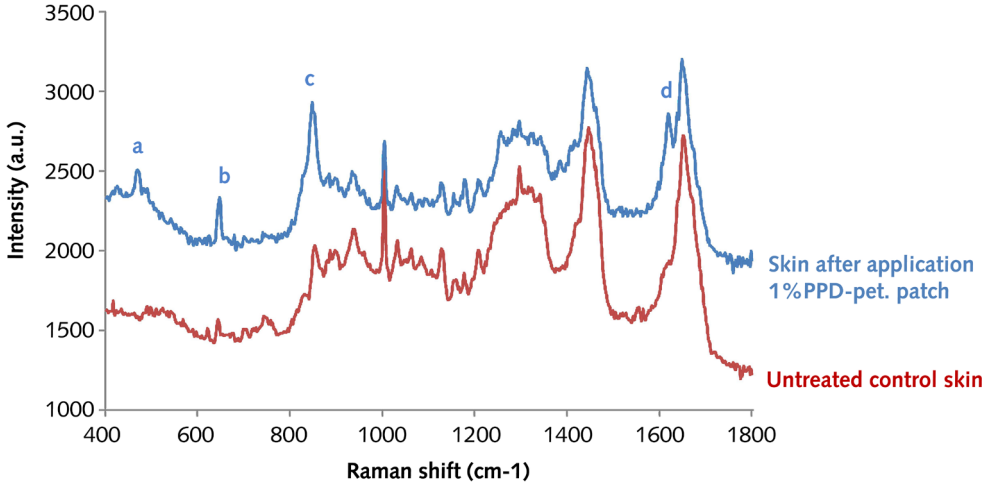

Figure 2 Examples of Raman spectra of untreated control skin and skin treated with 1%PPD-petrolatum for 2 h 40 min, recorded at a depth of 8 micron. In comparison with the untreated skin, the spectrum of the treated skin contained peaks characteristic to PPD (marked with a, b, c and d). No peaks of petrolatum, or the PPD derivatives were observed.

Detection of PPD and its derivatives in the living epidermis

We were interested in the influence of application time on the identified signals of PPD and subsequent formation of PPD derivatives in the living epidermis. Therefore, a range of application-time experiments were performed. In Figure 3, the amount of PPD (mg PPD/g keratin) is plotted against the depth. The colored graphs represent the signals for different application times (30 min, 2 h, 2 h 40 min, 3 x 1 h, 23 h) of the 1% PPD-petrolatum patch. Approximately 5-10 repeated profiles were measured in 1 volunteer. Directly after removal of the patch, the relative amounts of PPD in the skin were highest after repeated application of 3 x 1 h (350 mg PPD/g keratin) followed by single applications for 2 h 40 min, 2 h, and 23 h. These differences became less apparent deeper in the skin. There is no clear indication that PPD also penetrated deeper into the skin after a longer application time. After the application of 30 minutes, already substantial amounts of PPD (approximately 70 mg PPD/g keratin at 4 µm depth) were detectable in the skin. Thus, immediately after removal of the patch, PPD was detectable up to approximately 20 µm below the skin surface. Here, the amount of PPD reaches its lower detection limit of 13 mg PPD/g keratin (1.3 mass %). Interestingly, the amounts of PPD after 23 h application of 1% PPD-petrolatum were comparable to that of the 30 min application. For readability purposes, the combined standard deviation (SD; in one direction) is only shown for the upper graph. The SD of the remaining curves was similar.

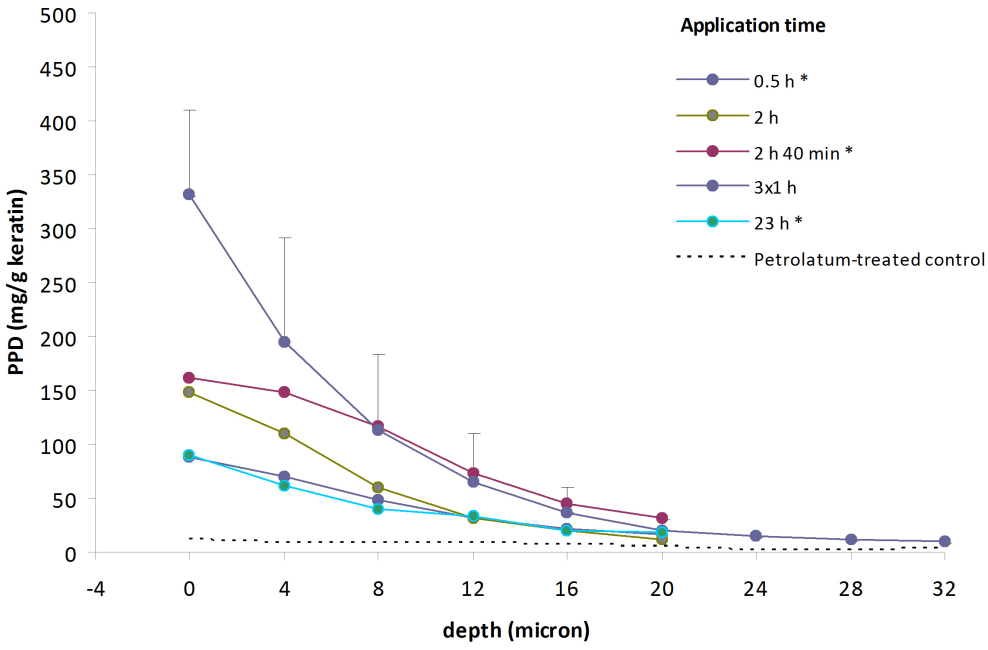

Figure 3 PPD content in skin (mg/g keratin) immediately after removal (0-5 min) of 1%PPD-petrolatum patch, after different application times. * Measured in only 1 volunteer. Per depth 5-10 measurements were averaged.

PPD clearance as a function of time

PPD clearance was monitored after application times of 2 h and 3 x 1 h. Follow-up measurements were performed at different times after removal of the patch (0.5-7 h). Figures 4 and 5 display the PPD content (mg PPD/g keratin) as a function of distance to the skin surface. Each curve is the average of 5-20 profiles per volunteer. Evidently, the PPD contents in the skin decreases with time, most clearly seen between depths of 0-10 µm. The PPD content approaches the baseline reading (black dashed line Figures 4 and 5) at about 20 µm below the skin surface. The SD is shown for the 0-0.5 h graph only. SDs for the other curves were comparable. None of the measurements detected penetrated petrolatum beyond the top few micrometers of the skin. Based on a 50% decrease of the AUC after application of the 3 x 1 h patch (AUC calculated from Figure 5), the half-time of PPD was estimated at 3 h.

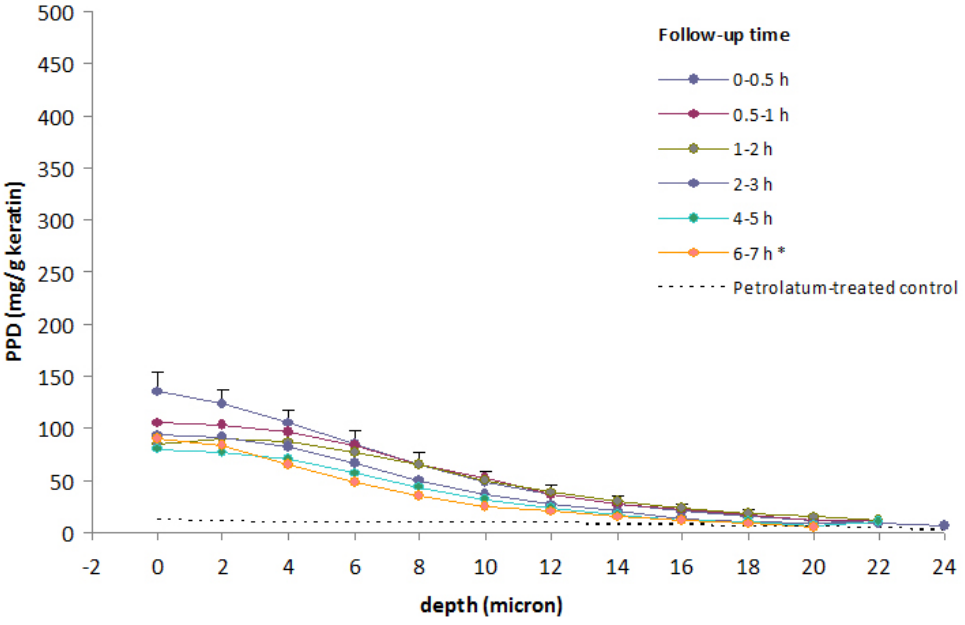

Figure 4 PPD content in skin (mg/g keratin) at several time intervals after removal of 1% PPD-petrolatum patch applied for 2 hours. * Measured in only 1 volunteer. Per depth 10-20 measurements were averaged.

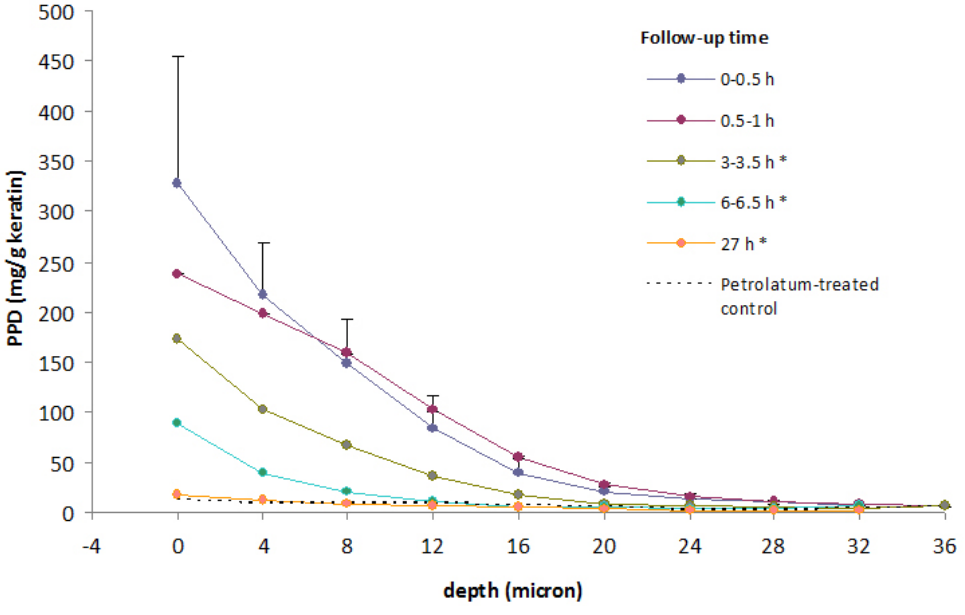

Figure 5 PPD content in skin (mg/g keratin) at several time intervals after removal of 1% PPD-petrolatum patch applied for 3 x 1 hour. * Measured in only 1 volunteer. Per depth 5-20 measurements were averaged.

Detection of *N*-acetylated PPD and its oxidation products.

All spectra collected during the experiments were inspected for signals of BB, MAPPD and DAPPD. Although PPD was clearly present, no characteristic Raman signals of these PPD derivatives could be detected in the skin. The exact location(s) of *N*-acetylation within the epidermis is (are) not known. Based on *in vitro* results originated from non-differentiated keratinocytes [17] we expected to find *N*-acetylation in deeper layers of the viable epidermis. Measurements specifically aiming at the upper viable epidermis 30 µm below the skin surface showed small amounts of PPD. However, none of the reference spectra of BB, MAPPD and DAPPD could be distinguished. Thus, neither the stratum corneum nor the viable epidermis showed detectable amounts of BB, MAPPD or DAPPD.

Discussion

With this study we primarily aimed to get insight into the *in vivo* penetration and clearance of PPD in the human skin. We have demonstrated that measuring the penetration of PPD into the living human skin is possible. Application of a 1% PPD-petrolatum patch for 30 min revealed that PPD penetrates the stratum corneum relatively fast and in substantial amounts. This is consistent with data from dose-time relationship studies, where even shorter application times (5 and 15 min) of a 1% PPD-petrolatum patch were sufficient to elicit allergic reactions in 16% and 38% of the PPD sensitized subjects,[18,19] thereby indicating that PPD had penetrated at least into the viable epidermis. Although our measurements immediately after removal of the patch showed that a longer application time resulted in higher amounts of PPD in the skin, this was not observed for the 23 h application in which the PPD content in the skin approached the amount found after a 30 min patch application (Figure 3). A probable explanation is that after certain application times (between 2 h 40 min and 23 h) the layer of PPD cream closest to the skin surface is depleted of PPD. This may call into question the gold standard of 48 h application in diagnostic patch testing.[20] Since PPD is soluble in water, it is conceivable that PPD first has to dissolve in water before it can penetrate the skin. Inspection of 1% PPD in petrolatum by regular light microscopy indeed revealed the PPD present as crystals. This would imply that PPD primarily penetrates from the interface layer of the patch which directly contacts the (aqueous) skin. Therefore, the amount of PPD available for penetration may deplete rapidly. Re-application of a fresh patch every hour (3 x 1 h) aimed at applying an infinite dose of PPD available for penetration.

The clearance experiments of both the 2 h and the 3 x 1 h application showed that PPD content decreases over time. The 3 x 1 h patch resulted in 2-3 times more PPD in the skin as compared to the 2 h patch (at comparable follow-up measurements of 0-0.5 h and 0.5-1 h). Raman studies on the application of more lipophilic substances such as Metronidazole and *trans*-retinol (dissolved in propylene glycol) showed that with time, they penetrated deeper into

the skin.[13,14] In our study the total penetration depth did not increase with time. Clearance of PPD from the stratum corneum due to passive diffusion into the viable epidermis and deeper layers, might be accompanied by disappearance of PPD by conversion into the aforementioned PPD derivatives BB, MAPPD and DAPPD. However, in this pilot-study we have not been able to detect any of these PPD derivatives. Various studies have demonstrated the formation and presence of acetylated PPD derivatives in skin samples (reviewed by Pot *et al*. 2013).[2] A possible explanation for the absence of these compounds in the current study is that formation of derivatives result in concentrations below the limit of detection of the experimental design. In a study by Hu *et al*.,[21] using human reconstructed epidermis, the concentration of PPD derivatives in skin (tissue homogenates) detected by liquid chromatography-mass spectrometry was also below the limit of detection. Although PPD derivatives could be detected in the culture medium, it is assumed that this is the result of accumulation in the closed compartment of the culture medium. In their study, Hu *et al*. used an application time of 30 minutes and a dose of 36.7 μg cm^{-2} PPD (dissolved in buffer) and a 23.5 h follow-up period. The PPD dose applied in our Raman study was considerably higher (approximately 312.5 μg cm^{-2} PPD), yet it cannot be excluded that this amount of PPD has led to NAT1-enzyme saturation and inhibition of *N*-acetylation capacities and thereby to a reduced amount of acetylated PPD derivatives.[17,21]

Conclusion

We have monitored *in vivo* penetration of PPD into the human skin by Raman spectroscopy. Thereby, a clear time- and depth dependent decrease of the local content of PPD in the stratum corneum could be demonstrated. Detection of PPD was possible within the stratum corneum and the viable epidermis. Within the design of our pilot-study, oxidized or acetylated PPD derivatives could not be detected.

References

1. Jahn S, Faber H, Zazzeroni R et al. Electrochemistry/mass spectrometry as a tool in the investigation of the potent skin sensitizer p-phenylenediamine and its reactivity toward nucleophiles. Rapid Commun Mass Spectrom 2012; 26:1453-64.
2. Pot LM, Scheitza SM, Coenraads PJ et al. Penetration and haptenation of p-phenylenediamine. Contact Dermatitis 2013; 68:193-207.
3. Aeby P, Sieber T, Beck H et al. Skin sensitization to p-phenylenediamine: the diverging roles of oxidation and N-acetylation for dendritic cell activation and the immune response. J Invest Dermatol 2009; 129:99-109.
4. White JM, Kullavanijaya P, Duangdeeden I et al. p-Phenylenediamine allergy: the role of Bandrowski's base. Clin Exp Allergy 2006; 36:1289-93.
5. Eilstein J, Gimenez-Arnau E, Duche D et al. Synthesis and reactivity toward nucleophilic amino acids of 2,5-[13C]-dimethyl-p-benzoquinonediimine. Chem Res Toxicol 2006; 19:1248-56.
6. Eilstein J, Gimenez-Arnau E, Duche D et al. Sensitization to p-amino aromatic compounds: Study of the covalent binding of 2,5-dimethyl-p-benzoquinonediimine to a model peptide by electrospray ionization tandem mass spectrometry. Bioorg Med Chem 2008; 16:5482-9.
7. Coulter EM, Jenkinson C, Wu Y et al. Activation of T-cells from allergic patients and volunteers by p-phenylenediamine and Bandrowski's base. J Invest Dermatol 2008; 128:897-905.
8. Steiling W, Kreutz J, Hofer H. Percutaneous penetration/dermal absorption of hair dyes in vitro. Toxicol In Vitro 2001; 15:565-70.
9. Hueber-Becker F, Nohynek GJ, Meuling WJ et al. Human systemic exposure to a [14C]-para-phenylenediamine-containing oxidative hair dye and correlation with in vitro percutaneous absorption in human or pig skin. Food Chem Toxicol 2004; 42:1227-36.
10. Dressler WE, Appelqvist T. Plasma/blood pharmacokinetics and metabolism after dermal exposure to para-aminophenol or para-phenylenediamine. Food Chem Toxicol 2006; 44:371-9.
11. Goebel C, Coenraads PJ, Rothe H et al. Elicitation of the immune response to p-phenylenediamine in allergic patients: the role of dose and exposure time. Br J Dermatol 2010; 163:1205-11.
12. Caspers PJ, Lucassen GW, Carter EA et al. In vivo confocal Raman microspectroscopy of the skin: noninvasive determination of molecular concentration profiles. J Invest Dermatol 2001; 116:434-42.
13. Tfayli A, Piot O, Pitre F et al. Follow-up of drug permeation through excised human skin with confocal Raman microspectroscopy. Eur Biophys J 2007; 36:1049-58.
14. Pudney PD, Melot M, Caspers PJ et al. An in vivo confocal Raman study of the delivery of trans retinol to the skin. Appl Spectrosc 2007; 61:804-11.
15. Broding HC, van der Pol A, de Sterke J et al. In vivo monitoring of epidermal absorption of hazardous substances by confocal Raman micro-spectroscopy. J Dtsch Dermatol Ges 2011; 9:618-27.
16. Kawakubo Y, Merk HF, Masaoudi TA et al. N-Acetylation of paraphenylenediamine in human skin and keratinocytes. J Pharmacol Exp Ther 2000; 292:150-5.

17. Bonifas J, Scheitza S, Clemens J et al. Characterization of N-acetyltransferase 1 activity in human keratinocytes and modulation by para-phenylenediamine. J Pharmacol Exp Ther 2010; 334:318-26.
18. Hextall JM, Alagaratnam NJ, Glendinning AK et al. Dose-time relationships for elicitation of contact allergy to para-phenylenediamine. Contact Dermatitis 2002; 47:96-9.
19. McFadden JP, Wakelin SH, Holloway DB et al. The effect of patch duration on the elicitation of para-phenylenediamine contact allergy. Contact Dermatitis 1998; 39:79-81.
20. White JM. Patch testing: what allergists should know. Clin Exp Allergy 2012; 42:180-5.
21. Hu T, Bailey RE, Morrall SW et al. Dermal penetration and metabolism of p-aminophenol and p-phenylenediamine: application of the EpiDerm human reconstructed epidermis model. Toxicol Lett 2009; 188:119-29.